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FORMULATION AND EVALUATION OF PENTOXIFYLLINE LIPOSOME FORMULATION

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Pentoxifylline is a drug with narrow therapeutic index and short biological half-life. This study aimed at developing and optimizing liposomal formulation of Pentoxifylline in order to improve its bioavailability. In evaluation study the effect of the varying composition of lipids on the properties such as encapsulation efficiency, particle size and drug release were studied. Phase transition study was carried out to confirm the complete interaction of pentoxifylline with bilayer structure of liposome. Moreover, the release of the drug was also modified and extended over a period of 8 h in all formulations. F1 emerged as the most satisfactory formulation in so far as its properties were concerned. Further, release of the drug from the most satisfactory formulation (F1) was evaluated through dialysis membrane to get the idea of drug release. The mechanism of dug release was governed by Peppas model.

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1. Introduction

The main objective of drug delivery systems is to deliver a drug effectively, specifically to the site of action and to achieve greater efficacy and minimise the toxic effects compared to conventional drugs. Liposomal vesicles were prepared in the early years of their history from various lipid classes identical to those present in most biological membranes. Basic studies on liposomal vesicles resulted in numerous methods of their preparation and characterization [(1)]. Liposomes are broadly defined as lipid bilayers surrounding an aqueous space. Multilamellar vesicles (MLV) consist of several (up to 14) lipid layers (in an onion-like arrangement) separated from one another by a layer of aqueous solution. These vesicles are over several hundred nanometers in diameter. Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25–50 nm (according to some authors up to 100 nm) in diameter. Large unilamellar vesicles (LUV) are, in fact, a very heterogenous group of vesicles that, like the SUVs, are surrounded by a single lipid layer. The diameter of these liposomes is very broad, from 100 nm up to cell size [(2)]. Besides the technique used for their formation the lipid composition of liposomes is also, in most cases, very important. For some bioactive compounds the presence of net charged lipids not only prevents spontaneous aggregation of liposomes but also determines the effectiveness of the entrapment of the solute into the liposomal vesicles. Natural lipids, particularly those, with aliphatic chains attached to the backbone by means of ester or amide bonds (phospholipids, sphingolipids and glycolipids) are often subject to the action of various hydrolytic (lipolytic) enzymes when injected into the animal or human body. These enzymes cleave off acyl chains and the resulting lysolipids have destabilising properties for the lipid layer and cause the release of the entrapped bioactive component(s). As a result new types of vesicles, that should

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merely bear the name of liposomes as their components are lipids only by similarity of their properties to natural (phospho)lipids, have been elaborated. These vesicles, liposomes, are made of various amphiphile molecules (the list of components is long). The crucial feature of these molecules is that upon hydration they are able to form aggregation structures resembling an array and have properties of natural phospholipid bilayers [(3)].

Pentoxifylline is widely used as vasodilator in peripheral and cerebral vascular disorder to inhibit the production of cytokines tumour necrosis alpha factor thus, used in metastasis. Pentoxifylline is a PDE4 inhibitor increasing intracellular cAMP. It also acts as inhibitor of tumour necrosis factor-alpha. It is a drug of choice for vasodilation and metastasis. But it has several drawbacks such as narrow therapeutic index, short biological half-life [(4)]. These factors necessitated liposomal formulation for pentoxifylline. As this dosage form would reduce the dosing frequency hence better patient compliance.

Phospholipids such as phosphatidylcholine and cholesterol were selected for the formation of liposomes into which the drug was incorporated. Cholesterol incorporated into phospholipids membranes in very high concentration up to 1:1 or 2:1 molar ratio [(5)]. Cholesterol acts as a 'fluidity buffer' since below the phase transition it tends to make membrane less ordered while above transition it tends to make membrane more ordered thus suppressing the tilts and shifts in membrane structure specifically at phase transition.

The present study is aimed with the formulation of liposomes of pentoxifylline followed by the evaluating parameters such as encapsulation efficiency, particle size, phase transition study and *in vitro* drug release.

2. Materials and method

Pentoxifylline was obtained as a gift sample from Bakul Pharma Pvt. Ltd., Mumbai. Phosphatidylcholine was obtained as gift sample from Perfect Biotech, Nagpur. Cholesterol was purchased from Loba chemie. All the other chemicals, reagents and solvents used like potassium chloride, sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide pellets, acetone, chloroform, and methanol were of analytical reagent grade.

A. Evaluation of raw materials

Identification and standardization of drug and other excipients were carried out as per the official procedures mentioned in respective monographs.

B. Preparation of liposomes

Liposomes were prepared by physical dispersion method using different ratio of lipids. In this method the lipids were dissolved in chloroform. This solution of lipids in chloroform was spread over flat bottom conical flask. The solution was then evaporated at room temperature without disturbing the solution. The hydration of lipid film form was carried out with aqueous medium phosphate buffer (pH 7.4). For this the flask was inclined to one side and aqueous medium containing drug to be entrapped was introduced down the side of flask and flask was slowly returned to upright orientation. The fluid was allowed to run gently over lipid layer and flask was allowed to stand for 2 h at 37^0C for complete swelling. After swelling, vesicles are harvested by swirling the contents of flask to yield milky white suspension. Then formulations were subjected to centrifugation. Different batches of liposomes were prepared in order to select an optimum formula. All batches of liposomes were prepared as per the general method described above and composition of lipids for the preparation of liposomes is given in Table 1.

Table 1. Composition of lipids for preparation of liposome.

Sr. No	Formulation No.	Phosphatidylcholine	Cholesterol
		PARTS	
1	F1	9	1
2	F2	8	2
3	F3	7	3
4	F4	6	4
5	F5	5	5
6	F6	4	6

Each formulation contain 200 mg of drug

Table 2. Optimized formula for liposome preparation

Sr. No.	*Constituents*	*Quantity*
1	Phosphatidylcholine	270 mg
2	Cholesterol	30 mg
3	Solvent(Chloroform)	5 ml
4	Drug(Pentoxifylline)	200 mg
5	Phosphate buffer pH 7.4	10 ml

C. Drug entrapment efficiency of liposomes

Entrapment efficiency of liposomes was determined by centrifugation method. Aliquots (1 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (Remi R4C) at 3500 rpm for a period of 90 min [6]. The clear supernatants were removed carefully to separate non-entrapped pentoxifylline and absorbance recorded at 274 nm. The sediment in the centrifugation tube was diluted to 100 ml with phosphate buffer pH 7.4 and the absorbance of this solution was recorded at 274 nm.

Amount of pentoxifylline in supernatant and sediment gave a total amount of pentoxifylline in 1 ml dispersion.

% entrapment of drug was calculated by the following formula:

$$\textbf{\% Drug Entrapped (PDE)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100$$

D. Particle size analysis

The particle size of liposomes was determined by using motic digital microscope model No. DMW. All the prepared batches of liposomes were viewed under microscope to study their size [7]. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined. Particle size of liposomes was shown in Table 3.

Table 3: Evaluation parameters of liposome.

SR. NO	FORMULATION NO.	% DRUG ENTRAPPED	MEAN PARTICLE SIZE µm± SD
1	F1	48.92 ± 0.81	6.24 ± 0.09
2	F2	47.23 ± 0.92	7.14 ± 0.098
3	F3	44.71 ± 0.53	10.74 ± 0.064
4	F4	40.51 ± 1.02	12.27 ± 0.082
5	F5	35.28 ± 1.07	15.07 ± 0.105
6	F6	29.64 ± 0.63	14.03 ± 0.051

(Mean ± S.D., n=3)

E. Phase transition study

Differential scanning calorimetry (DSC) thermograms of the phosphatidylcholine, cholesterol and pentoxifylline were recorded on a differential scanning calorimeter. Thermograms of both blank and pentoxifylline liposomal dispersions were recorded individually. The liposomal dispersions were weighed in an aluminium cuvette and sealed with an aluminium lid. The cuvette was placed in the DSC and heated from 20^0C to 200^0C at a heating rate of 10^0C/min in nitrogen atmosphere. The scan was recorded and plotted showing heat flow (w/g) on the Y-axis and temperature (^{0}C) on the X-axis.

F. *In Vitro* drug release study

The release studies were carried out in 250 ml beaker containing 100 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 250 ml beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped pentoxifylline, liposome dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. Aliquots were withdrawn (5 ml) at specific intervals, filtered and the apparatus was immediately replenished with same quantity of fresh buffer medium.

3. Results and discussion

Among the various methods, physical dispersion method is widely used to prepare liposomes. This method yields the liposomes with a heterogeneous size distribution. Also the liposomes that are formed are multilamellar in nature.

The result of drug entrapment efficiency of liposomes (Table 3) indicates that as the concentration of phosphatidylcholine decreases, drug entrapment efficiency of liposomes decreases which was due to the saturation of lipid bilayer with reference to the drug where low phosphatidylcholine content provides limited entrapment capacity. The encapsulation efficiency of liposomes is governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Cholesterol improves the fluidity of the bilayer membrane and improves the stability of bilayer membrane in the presence of biological fluids such as blood/plasma.

From results of % drug entrapped it was observed that as the percentage of cholesterol increased there was subsequent increase in the stability and rigidity of liposomes but at the same time percentage drug entrapment reduced due to reduction in phosphatydilcholine.

Results of particle size analysis showed that, as the concentration of cholesterol increases particle size increases which was may be due to formation of rigid bilayer structure but this was up to a specific concentration as there was also decrease in size of formulation F6.

From phase transition study it was observed that DSC thermograms of blank unloaded liposomal dispersion shift in the melting endotherm of cholesterol from 150.01^0C to 125.17^0C and for Phosphatidylcholine from 42.64^0C to 72.2^0C signifying that all the lipid components interact with each other to a great extent while forming the lipid bilayer. In the DSC thermogram of pentoxifylline liposomal dispersion, Batch F1, the cholesterol endotherm exhibited a shift from 150.01^0C to 133.53^0C while the Phosphatidylcholine endotherm was found to be shifted from 42.64^0C to 73.44^0C. Absence of the melting endotherm of pentoxifylline suggested significant interaction of pentoxifylline with the bilayer structure. The phase transition temperature gives good clues about liposomal stability, permeability and whether a drug is entrapped in the bilayer or in the aqueous compartments.

In Vitro drug release of various formulations of liposomes are shown in Figure 1.

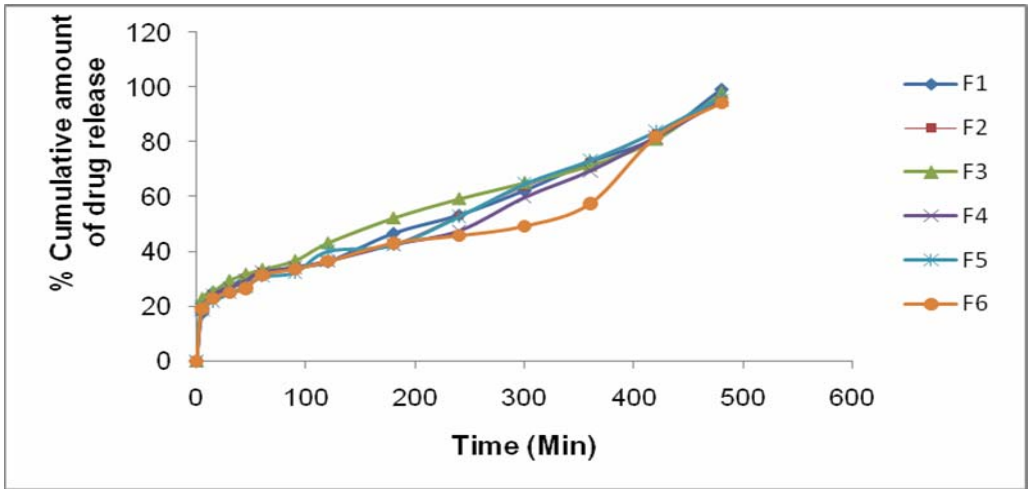

Fig. 1. In vitro drug release of various formulations.

The study of drug release kinetics showed that majority of the formulations governed by peppas model. The curve was obtained after plotting the cumulative amount of drug released from each formulation vs. time.

All the formulations showed release up to 8 h and above 90% of drug released with each formulation. Formulation F1 (99.23%) showed maximum release while other formulation showed less amount of drug release in 8 h. Formulation F1 has highest correlation coefficient (r =0.9552) value and follows drug release by peppas model.

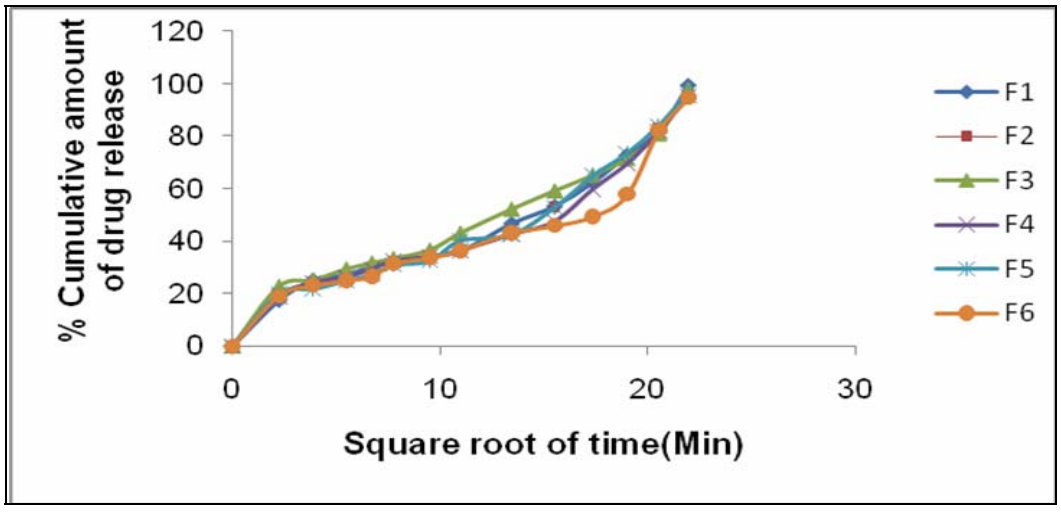

Fig. 2: Higuchi's plot

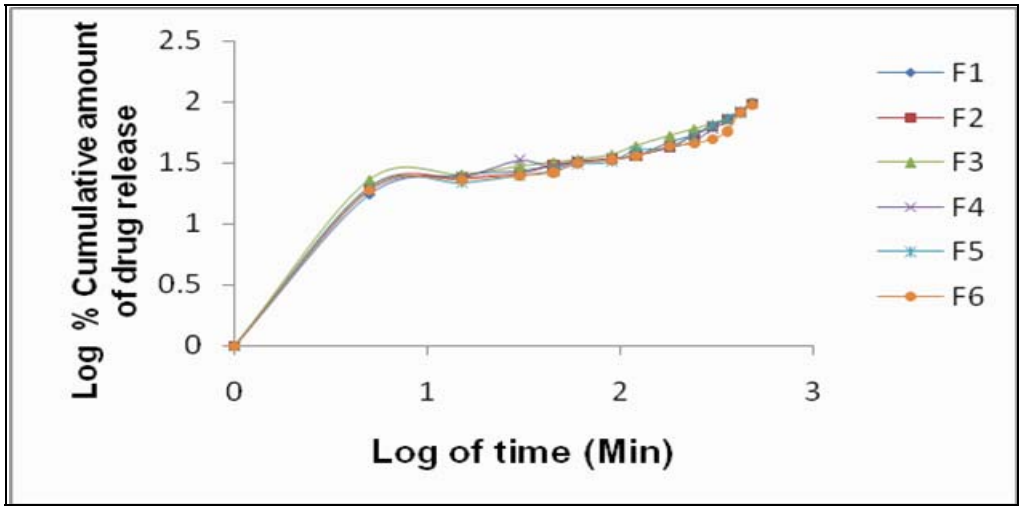

Fig. 3: Peppas korsmeyer's plot

4. Conclusion

The present study demonstrated the successful preparation of pentoxifylline liposomes and its evaluation. Formulation F1 showed high encapsulation efficiency with minimum particle size and drug release over an 8 h, hence suppose to give greater bioavailability and considered as good liposomal formulation.

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